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Enriched Accumulation and Biotransformation of Selenium in the Edible Seaweed *Laminaria japonica*

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Accumulations of selenium in kelp *Laminaria japonica* cultured in seawater was achieved by adding selenite (Na_2SeO_3) with or without N−P ($NaNO_3$ + NaH_2PO_4) nutrients at different concentrations. Biotransformation of selenium in the kelp was investigated through measuring the selenium of biological samples and different biochemical fractionations. The results showed that the optimal selenite-enrichment concentration is 200 mg L^{-1}, which can allow the kelp to accumulate a total selenium content from 0.51 ± 0.15 to 26.23 ± 3.12 μg g^{-1} of fresh weight (fw). Selenium composition analysis of kelp (control group) showed that selenium is present as organic selenium, which is up to 86.22% of the total selenium, whereas inorganic selenium is barely 4.85%. When *L. japonica* was exposed for 56 h in seawater containing 200 mg L^{-1} Na_2SeO_3, the organic selenium was 16.70 μg g^{-1} of fw (68.23%) and inorganic selenium was 4.71 μg g^{-1} of fw (19.26%). The capability of accumulation of selenium was further enhanced by adding N−P nutrients to the selenite-enriched medium. Total selenium is increased to be 33.65 μg g^{-1} of fw at optimal concentration of N−P nutrient (150 mg L^{-1} $NaNO_3$ and 25 mg L^{-1} NaH_2PO_4), whereas the inorganic selenium was not increased and remained at 4.597 μg g^{-1} of fw (13.36%), and the increased part of selenium was organic selenium. This implied that kelp *L. japonica* could effectively transform inorganic selenium into organic selenium through metabolism.

KEYWORDS: Selenium; seaweed; accumulation; biotransformation

INTRODUCTION

Selenium (Se) is an essential trace element indispensable for normal functioning of the body, as a component of glutathione peroxidases primarily responsible for reducing peroxide free radicals and prostaglandin synthesis by protecting the oxidative state of lipid intermediates (*1*). In most cases, the nutritional supply of Se depends on the concentration of Se in foods, and the soil selenium content determines the amount of selenium concentrated in plant sources, which can vary as much as 200-fold between the same crops grown in different regions. Although most people probably do not take in enough selenium, gross deficiencies are rare in Western countries, but soils in some areas are selenium deficient, and people who eat foods grown primarily on them are at risk for deficiency. In selenium-deficient regions of China, selenium deficiency results in some endemic diseases, such as Keshan disease (cardiomyopathy) and Kashin−Beck disease (chondrodystrophy) (*2*, *3*). Selenium supplementation can correct this type of deficiency. In addition, a low concentration of selenium in plasma has been identified as a risk factor for several diseases, including certain types of cancer, cardiovascular disease, osteoarthritis, and AIDS (*4*−*6*). Selenium fortification is becoming popular as a supplement for a nutritional demand for chemoprevention notwithstanding controversy, and several large-scale selenium supplementation trials with humans are now underway (*7*, *8*). Selenium-enriched foods have been developed including fruits, wheat, brewer's yeast, and vegetables such as tomato, broccoli, onions, and garlic cultured in selenite-enriched soil or medium (*9*−*13*). However, the potential selenium accumulation in soil could lead to contamination through irrigation (*14*, *15*).

In addition, selenium has a synergistic effect with iodine to keep the thyroid function active. Combined selenium and iodine deficiency leads to myxedematous cretinism (*16*). In China, there is a long history of use of the edible seaweed *Laminaria japonica* to treat iodine deficiency disorder, because this marine macroalga contains high amounts of iodine (*17*). Nevertheless, the selenium content in this seaweed is very low (*18*). Thus, we are trying to develop an enrichment method to make the kelp a health food and feed rich in both iodine and selenium. In this paper, the results of accumulation and biotransformation of selenium in the kelp *L. japonica* are presented.

MATERIALS AND METHODS

Seaweed Material. The kelp *L. japonica* was collected in April 2002 from a marine farming factory in Shandong Province. The seaweeds chosen for experiment were of the same size and growth stage. All

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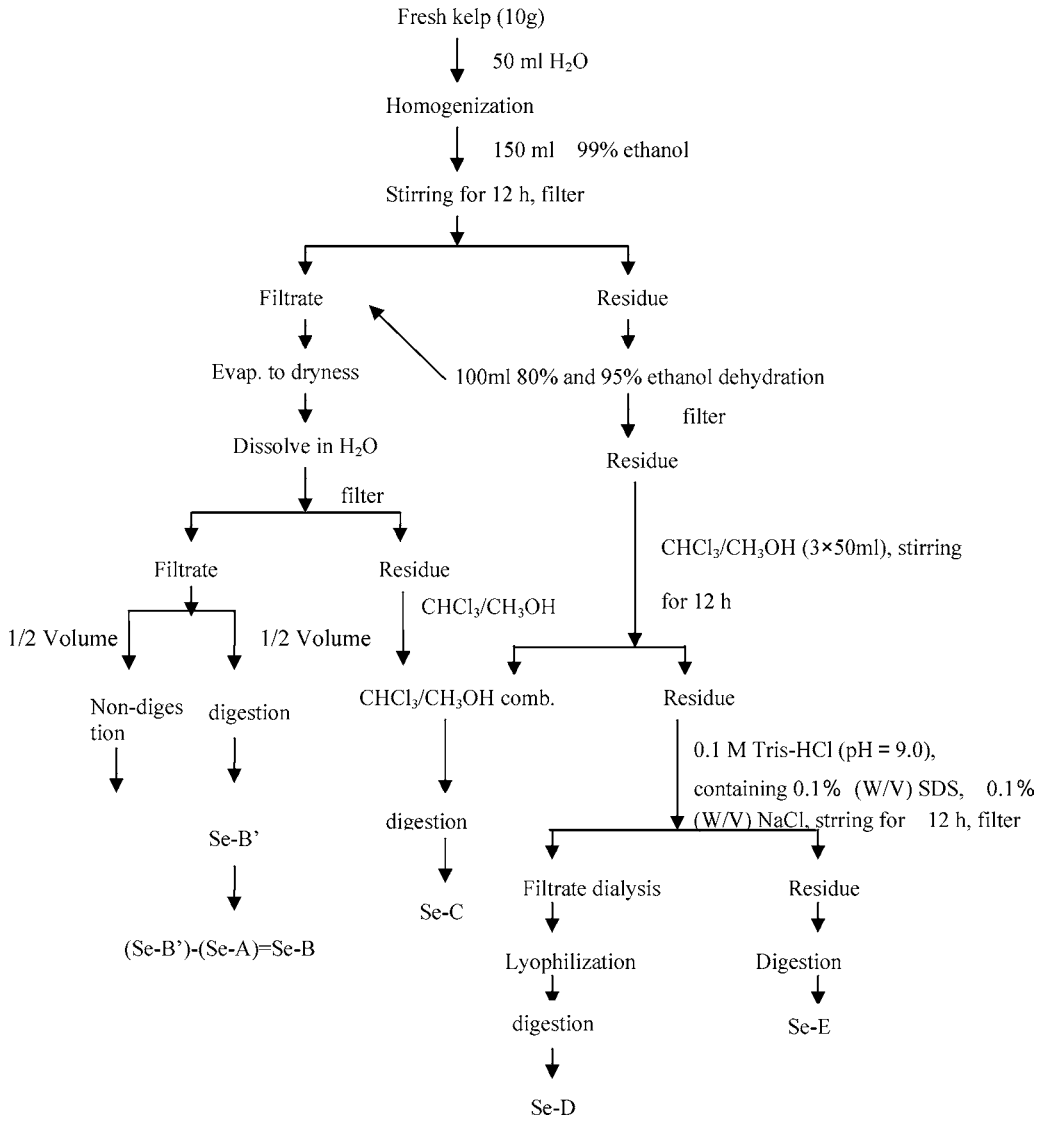

Figure 1. Separation procedure of selenium biochemical fractions in kelp.

samples were washed with filtered seawater to remove epiphytes, sand, and any debris.

Detection of the Optimal Concentration of Selenite to Accumulation. The living kelp *L. japonica* samples in rapid growth stage with an average length of 2 m were soaked in a transparent plastic container full of seawater culture medium (10 L, two kelps in one container) enriched with sodium selenite of 0, 30, 60, 200, 350, 500, and 1000 mg L^{-1}, respectively. Each group was in triplicate. Short-term culture was carried out under natural sunlight (160 μmol m^{-2} s^{-1}) at a temperature of 18 °C. All of the samples (5 g of the mid part) were taken out after 56 h to determine the selenium content. On the basis of the results, the concentration of sodium selenite at 200 mg L^{-1} was chosen for further experimentation. Furthermore, the selenium contents in different parts (based on the shape of kelp, upper part, mid part, and basal part) of this group were determined also to make the following sampling easier. Accordingly, a 5 g sample from the mid part was chosen for selenium measurement in the other experiments.

Determination of Kinetic Accumulation of Selenite. The seaweeds were soaked in the transparent plastic container full of seawater culture medium enriched with sodium selenite of 200 mg L^{-1}, with the same culture conditions as above. The seaweed samples (5 g each) were collected at different time periods of 1, 2, 3, 6, 12, 24, 32, 48, and 56 h, respectively.

Influences of Nitrogen and Phosphorus Nutrition on Selenite Accumulation. Effects of adding the nutrients nitrogen and phosphorus were investigated by adding $NaNO_3$ and NaH_2PO_4 with different concentrations into the above selenite-enriched seawater medium (selenite was kept at 200 mg L^{-1}). The seaweed samples (5 g each) were collected at the 56 h point in the culture period to determine their selenium contents.

Biochemical Fraction of Kelp Accumulated with Selenite. On the basis of the total selenium content determination results, three groups of kelp were chosen for the analysis of the speciation of selenium. These were a control group (assigned as $kelp_{control}$) without selenium enrichment, a simple selenium-enrichment group ($kelp_{Se}$) with selenite enrichment at 200 mg L^{-1}, and a nutrient-enrichment group ($kelp_{Se-N-P}$) with the highest total selenium content (data shown in **Table 3**). The separation procedure for each biochemical fraction is modified from that of Maher (*19*) and described in **Figure 1**. The different biochemical fractions include inorganic selenium (Se-A), small aqueous organic selenium (Se-B), lipid-bound selenium (Se-C), protein selenium (Se-D), and polysaccharide selenium (Se-E), isolated successively from 10 g of starting biological material. All of the chemical reagents were of analytical grade, and deionized water was used for all dilutions. Lyophilization was carried out using a model 18 freeze-dryer (Labconco).

Determination of Selenium by Graphite Furnace Atomic Absorption Spectrometry (GFAAS). Selenium was determined using a Varian model SpectrAA-220E graphite furnace atomic absorption spectrometer with a palladium solution (100 μg mL^{-1}) as matrix modifier (*20*). The instrument is composed of a graphite tube atomizer, a graphite furnace system (GTA-96), a programmable sample dispenser, and a data process station. The spectrometer was equipped with a

Table 1. Total Selenium Content in the Kelp at Different Selenite Concentrations (Micrograms per Gram of Fresh Weight)

	Na_2SeO_3						
	0 mg L^{-1}	30 mg L^{-1}	60 mg L^{-1}	200 mg L^{-1}	350 mg L^{-1}	500 mg L^{-1}	1000 mg L^{-1}
total Se[a]	0.51 ± 0.15	10.57 ± 1.89	21.65 ± 2.08	26.23 ± 3.12	24.44 ± 3.27	24.84 ± 2.88	21.22 ± 4.04
accumulation index[b]		20.7	42.5	51.4	47.9	48.7	41.6

[a] Data are presented as means ± SD. [b] The accumulation index is calculated as the mean value of total Se/0.51.

deuterium background corrector for the correction of nonspecific absorption signals.

The samples were diluted 1:5 with ascorbic acid and Triton-X solution before placement in the autosampler. Ascorbic acid Triton-X reagent was prepared as follows: 1 mL of Triton X-100 and 5 mL of 5% ascorbic acid solution were dispensed into a 100 mL volumetric flask and made up to volume with deionized water, followed by 5 min in an ultrasonic bath. Using the preinjection facility, the Pd modifier was introduced by the sampler into the graphite tube and dried in situ. The sample was then dispensed into the "prepared" tube and ashed. The selenium is thermally stabilized by the modifier up to the ashing temperature, and the matrix can be largely removed by filtration. For the total selenium and organic selenium analysis, samples (0.5 g for solid) were weighed in a Teflon vessel, and 1.0 mL of HNO_3 and 4.0 mL of hydrogen peroxide were added to perform the digestion. A microwave digester (CEM, MDS-2000) was used for heating the samples. The temperature program for selenium analysis starts with the injection into a preheated graphite tube. After drying, the sample is heated slowly to 600 °C, then to 1200 °C, and finally the atomization temperature was set at 2700 °C with palladium as the matrix modifier. A certified reference material (NCS GSBG 62029-90) with a certified selenium concentration of 1000 μg mL^{-1} was used to control the accuracy of total selenium determination. A fresh 100 ng mL^{-1} selenium standard was prepared daily in 0.5% hydrochloric acid from the selenium stock solution; a standard addition method was used for calibration with an average recovery rate at 98.5 ± 1.7%. The data from the automatic measurement were processed using quantitation software of the instrument.

RESULTS AND DISSCUSSION

Total Selenium at Different Selenite Concentrations. The total selenium content in the kelp without selenium enrichment is as low as 0.51 ± 0.15 μg g^{-1} of fw, which is comparable to the selenium content in Australian seaweeds (0.014−0.135 μg g^{-1} of dry wt in Phaeophyta, 0.153−0.434 μg g^{-1} of dry wt in Rhodophyta, and 0.053−0.264 μg g^{-1} of dry wt in Chlorophyta) (*19*). After the addition of selenite to the culture medium, the selenium concentration in the kelp increased rapidly from 10.57 ± 1.89 to 21.65 ± 2.08 μg g^{-1} of fw and reached the highest level of 26.23 ± 3.12 μg g^{-1} of fw, after which it decreased gradually when the selenite-enrichment concentration exceeded 350 mg L^{-1}. With a selenite concentration as high as 1000 mg L^{-1}, the normal physiological condition of kelp was disturbed and an unhealthy situation was noted. Results are shown in **Table 1**; the accumulation index in different selenite-enrichment concentrations ranged from 20.7 at an initial selenite-enrichment concentration of 30 mg L^{-1}, and the highest accumulation ratio is 51.4. Our aim in this experiment is to establish the optimal selenite concentration in culture medium to fulfill the selenium accumulation in the kelp, while not exposing too high a selenium concentration into the seawater. Therefore, 200 mg L^{-1} of selenite concentration has been selected for further experimentation as optimal for the new selenium-enrichment seaweed product development.

Total Selenium at Different Parts of the Kelp. Although the seaweed is a kind of lower plant without tissue differentiation as root, stem, and leaves as in higher plants, the kelp can be divided into upper part, mid part, and basal part because the overall appearance of the kelp is like a long leaf. The results from determining the selenium content in three different parts indicated that there is only slight difference (**Table 2**). The total selenium content in the control group (not treated with selenium-enriched medium) ranged from 0.45 ± 0.07 to 0.59 ± 0.12 μg g^{-1} of fw, with the highest concentration appearing in the upper part and from 20.56 ± 3.02 to 25.81 ± 1.35 μg g^{-1} of fw in the selenium-enrichment group (200 mg L^{-1}), with the highest concentration appearing in the mid part. The accumulation ratio showed more differences among each part, so we have selected the mid part as the biomaterial for selenium measurement in further experiments, because it has the highest ratio of 57.3.

Table 2. Total Selenium Content of Different Parts of the Kelp Plant (Micrograms per Gram of Fresh Weight)

sample	basal part	mid part	upper part
$kelp_{control}$[a]	0.55 ± 0.18	0.45 ± 0.07	0.59 ± 0.12
$kelp_{Se}$[a]	23.34 ± 2.33	25.81 ± 1.35	20.56 ± 3.02
accumulation index[b]	40.4	57.3	34.8

[a] Data are presented as means ± SD. [b] The accumulation index is calculated as the mean value of $kelp_{Se}$/mean value of $kelp_{control}$.

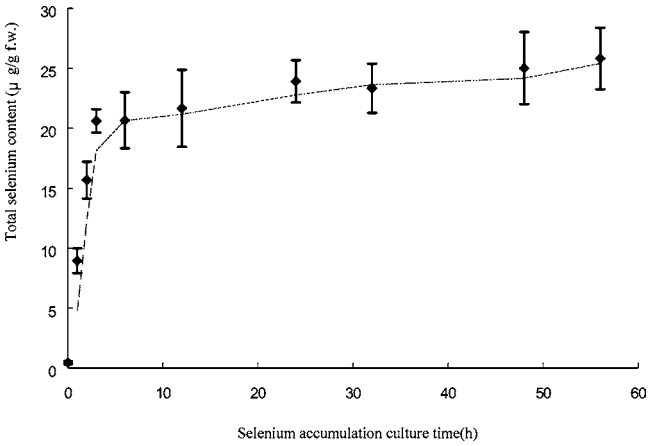

Figure 2. Total selenium content changes during different accumulation periods.

Total Selenium during Different Enrichment Periods. In our initial experiment, the enrichment culture period was 56 h because the kelp body can maintain its health during the whole process except for the highest selenium-enrichment group of 1000 mg L^{-1}. Nevertheless, one technical aspect in the selenium-enriched accumulation for kelp is the optimal time period to save time and manpower during this process without losing much of the accumulated selenium. In the experiment to determine the optimal period for selenium-enrichment culture, we find that the total selenium content increases rapidly for the first 3 h and then increases very slowly and almost maintains a stable level during the following hours (**Figure 2**). The selenium accumulation ratio is >50. The culture period of 3 h is considered to be feasible for a large-scale kelp selenium accumulation bioprocess. However, in our experiment, we still

Table 3. Total Selenium Content Changes Influenced by Nutrient Cofactors (Micrograms per Gram of Fresh Weight)

	$kelp_{Se-N-P}$							
	1	2	3	4	5	6	7	$kelp_{Se}$
$NaNO_3$ (mg L^{-1})	10	20	50	100	150	200	300	0
NaH_2PO_4 (mg L^{-1})	150	100	75	50	25	10	5	0
total Se[a]	18.03 ± 1.22	20.22 ± 1.80	19.89 ± 2.76	27.91 ± 3.15	33.01 ± 2.57	25.90 ± 3.35	26.19 ± 2.16	25.81 ± 1.39
accumulation index[b]	40.1	44.9	44.2	62.0	73.4	57.6	58.2	57.3

[a] Data are presented as means ± SD. [b] The accumulation index is calculated as the mean value of total Se/0.45 indicated in **Table 2**.

Table 4. Selenium Content Associated with Biochemical Fractions in the Kelp (Micrograms per Gram of Fresh Weight)

group	$kelp_{control}$	$kelp_{Se}$	$kelp_{Se-N-P}$
Se-A[a]	0.025 (4.85)	4.714 (19.26)	4.497 (13.36)
Se-B	0.118 (22.91)	0.540 (2.20)	2.138 (6.35)
Se-C	0.029 (5.63)	4.991 (20.39)	7.041 (20.92)
Se-D	0.245 (47.57)	7.388 (30.18)	10.730 (31.89)
Se-E	0.052 (10.11)	3.784 (15.46)	4.769 (14.17)
total Se	0.51	24.48[b]	33.65[c]
Se loss[d]	0.046 (8.93)	3.064 (12.51)	4.474 (13.30)

[a] The selenium biochemical fractions as Se-A, Se-B, Se-C, Se-D, and Se-E are inorganic selenium, small organic selenium, lipid selenium, protein-bound selenium, and polysaccharide-bound selenium, respectively. The detailed explanation can be traced in **Figure 1**. [b,c] The total selenium content in $kelp_{Se}$ and $kelp_{Se-N-P}$ is a little different from the data in **Tables 2** and **3** because the experiment was done with a different group. The kelp material used in $kelp_{Se}$ and $kelp_{Se-N-P}$ is selenite of 200 mg L^{-1} and selenite of 200 mg L^{-1}, $NaNO_3$ 150 mg L^{-1}, NaH_2PO_4 25 mg L^{-1}, respectively. [d] The selenium loss means the difference of [total Se − (Se-A) − (Se-B) − (Se-C) − (Se-D) − (Se-E)] in each group.

chose 56 h for all of the experiments to keep the results in different experimental groups comparable.

Improvement of Selenium Accumulation Efficiency by Using a Nutrient Cofactor. Because selenium accumulation happened very effectively in the living kelp body while not so visibly in the dead body (data not shown), we propose that the kelp may accumulate and transform selenium through metabolism. If this is the case, the nutrient cofactor should play a role. That means the selenium accumulation could be changed at different nutrient conditions to influence its metabolism. The results shown in **Table 3** clearly indicate that this hypothesis is correct. The addition of nitrogen and phosphorus as selenium accumulation cofactors is very effective in improving the efficiency. For example, at the same selenite concentration of 200 mg L^{-1} in the culture medium, the total selenium content almost doubles from 18.03 ± 1.22 to 33.01 ± 2.57 μg g^{-1} of fw between one group ($NaNO_3$ 10 mg L^{-1}, NaH_2PO_4 150 mg L^{-1}) and another ($NaNO_3$ 150 mg L^{-1}, NaH_2PO_4 25 mg L^{-1}). Riedel et al. point out during their study on selenium accumulation in fresh phytoplankton (*21*) that the high content of phosphorus possibly resists the selenium accumulation because of the chemical similarity between PO_4^{3-} and SeO_3^{2-}. Our results agree with this viewpoint. When the NaH_2PO_4 content ranged from 75 to 150 mg L^{-1}, the selenium content was lower than in the simple accumulation group (with the addition of only selenite in the seawater medium). However, the total selenium content at $NaNO_3$ of 150 mg L^{-1} and NaH_2PO_4 of 25 mg L^{-1} increases ~30% from 25.81 ± 1.39 to 33.01 ± 2.57 μg g^{-1} of fw, and the accumulation ratio reaches 73.4.

Chemical Speciation of Selenium and Biotransformation during the Accumulation Process. The results of selenium content in different biochemical fractions of kelp obtained from different treatment groups are shown in **Table 4**. The inorganic selenium content in the $kelp_{control}$ group is <5% of total selenium content; the major part is organic selenium, among which the protein-bound selenium is ~50% and aqueous small organic selenium compounds (most possibly as amino acid selenium) is ~20%. After inorganic selenium-enrichment culture, the selenium content in the kelp increases >50 times, and the inorganic selenium content increases 188-fold, from 0.025 to 4.714 μg g^{-1} of fw. Another comparable significant increase is lipid-bound selenium, from 0.029 to 4.991 μg g^{-1} of fw (172-fold), followed by polysaccharide-bound selenium (72-fold), protein-bound selenium (30-fold), and small aqueous organic selenium (5-fold), so that the distribution of selenium content in different biochemical fractions in the kelp $_{Se}$ group is much more even. Although the organic selenium part is still >80%, the aqueous small organic selenium increases only 5-fold, which indicates that the large increase in the inorganic selenite-enrichment culture is achieved through passive selenite permeation crossing the cell wall membrane and nonselective bonding onto lipid, protein, and polysaccharides. The small part of selenium accumulation is achieved by active biotransformation of selenite into aqueous small organic selenium possibly amino acid−selenium molecules.

In another nutrient-optimized group ($kelp_{Se-N-P}$), the inorganic selenium content is almost maintained static while the total selenium content increases from 24.48 to 33.65 μg g^{-1} of fw, which means that the increase during nutrient optimization is largely organic selenium, possibly through better metabolism and biotransformation processes. A detailed comparison between $kelp_{Se}$ and $kelp_{Se-N-P}$ indicated that the aqueous small organic selenium portion increased most, to as high as 4-fold (from 0.540 to 2.138 μg g^{-1} of fw), followed by protein-bound selenium (1.45-fold), lipid-bound selenium (1.41-fold), and polysaccharide-bound selenium (1.26-fold). On the basis of these data, it is suggested that the nitrogen and phosphorus can activate the selenium biotransformation metabolism by first increasing the synthesis of aqueous organic selenium (possibly amino acid selenium) from inorganic selenium (selenite in this case); then some part of the aqueous organic selenium molecules can be incorporated into seleno-protein synthesis, and some part is transformed into lipid-bound and polysaccharide-bound selenium.

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